Tolerance of high electrolytic and non-electrolytic osmolarities in *Bufo arenarum* premetamorphic tadpoles under organism density stress

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The mean (± standard error) lethal osmolarity was determined in electrolyte (NaCl) and non-electrolyte (mannitol) media for premetamorphic *Bufo arenarum* tadpoles. The effect of organism density stress over the survival capacity within high osmolarity media was evaluated. Acute toxicity tests were performed in accordance with the *Standard Methods* guidelines at two densities: high (1 tadpole per 4 ml of solution) and standard (1 tadpole per 20 ml of solution). Experimental solutions were obtained by adding NaCl and D-mannitol to distilled water. The osmolarity range ran from 141 to 271 mOsm. Control medium was artificial fresh water at 5 mOsm. There are no differences in survival between an electrolytic and a non-electrolytic medium and survival is not affected by high organism density. A mean lethal osmolarity was found at 279.0 ± 9.6 and 220.5 ± 2.0 mOsm for 48 and 144 h respectively.

The conditions under which the first life stages take place are of vital importance for the ecological success of a species. In general the aquatic environment under which most amphibians spend their larval stages is changing both in salinity, osmotic pressure, oxygen content and organism density, among other stress factors. The way in which some of these, and particularly organism density, may affect the growth and metamorphosis of *Bufo arenarum* tadpoles, has been widely studied (Murray, 1990; Miranda & Pisano, 1993; Kher, 1994). However, none of these works has taken into account the effect of osmotic stress combined with an increase in organism density.

Anuran tadpoles have registered tolerance at higher osmolarities than those of their natural medium. Except for a few species, tadpoles died when the internal medium became hyposmotic to the surroundings. Chemical composition of incubation media is not indifferent to tadpoles; because of their incapability to increase their plasmatic osmolarity by organic osmolytes synthesis, the compensatory response in hyperosmotic media appears to be almost entirely due to plasmatic NaCl (Balinsky, 1981). If the incubation medium is a non-electrolytic solution, hydric regulation is not possible and they react as limited osmocon-

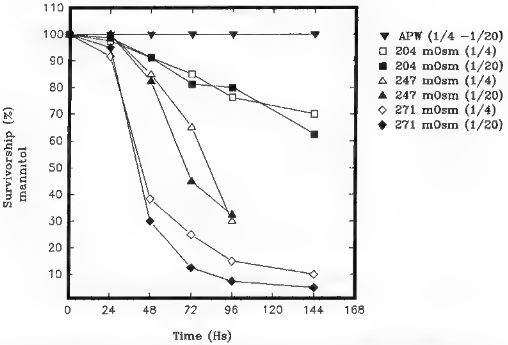

Fig. 1. Survivorship as a function of time for *Bufo arenarum* premetamorphic tadpoles (stage 26) exposed to different osmolarities of mannitol at 20° C and two conditions of density. 1/20: 1 tadpole/20 ml solution; 1/4 1 tadpole/4ml solution.

formers (Katz, 1987). In the specific case of *Bufo arenarum* tadpoles, exposed to osmotic stress, the existence of a dual behavior has been demonstrated, in which both highly saline environments are tolerated and survival in aionic media does not lead to important changes (Ferrari, 1995).

The aim of this work was to assess the survival response of *Bufo arenarum* tadpoles submitted to electrolytic and non-electrolytic osmolarity stress when they were exposed to organism density stress.

Semistatic assays were conducted following procedures of the American Public Health Association (Anonymous, 1992). Tadpoles obtained by "in vitro" fertilization were used at the first larval stage (Gosner, 1960). All tests were conducted with animals acclimatized 48 h before the beginning of the experiment at constant temperature (20°C) and photoperiod (12 L:12 D), and which remained under the same conditions throughout the experiments. All tests were conducted in duplicate at the rate of 1 larva/20 ml (1 g organism/l; Anonymous, 1992), and, to test the effect of density stress, 1 larva/4 ml (5 g organism/l).

The following osmolarities (in mOsm) were tested for electrolytic solutions (NaCl) as well as for non-electrolytic solutions (mannitol): 141, 204, 247 y 271. The control of artificial pond water (APW) of 5 mOsm was run (Alvarado & Johnson, 1966). Solutions were renewed daily. Tadpoles were examined at 24 h intervals during 144 h. Those exhibiting

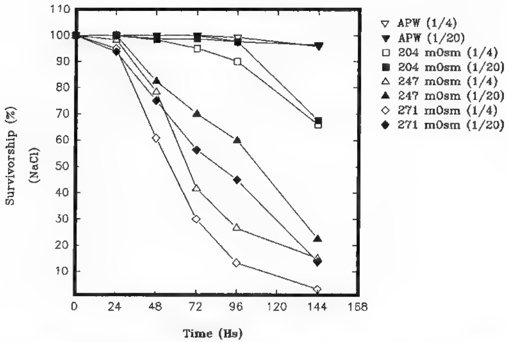

Fig. 2. Survivorship as a function of time for *Bufo arenarum* premetamorphic tadpoles (stage 26) exposed to different osmolarities of NaCl at 20°C and two conditions of density. 1/20: 1 tadpole/20 ml solution; 1/20: 1 tadpole/4ml solution.

no heartbeat or which did not respond to gentle prodding were considered dead and were removed from assay containers.

Mean lethal osmolarity (LO 50) was calculated by Probit analysis after adjusting for mortality among tadpoles in the control treatment with the Abbott's correction. Ninety-fice percent confidence limits for each estimate were calculated by Fieller's theorem (FINNEY, 1971).

Both in the NaCl as in the mannitol tests, in the two tested densities (1 tadpole/20 ml and 1 tadpole/4 ml) the mortality rate in controls (APW 5 mOsm) was always less than 5 %. Figure 1 shows the survival curves as a function of time for each of the non-electrolytic osmolarities (mannitol) assayed at both densities. The survival curves are similar at both densities. At 24 hours of exposure, mortality was less than 10 % in all the solutions, as of that moment mortality increased with osmolarity and time. At 271 mOsm, the fall in survival was very pronounced between 24 and 48 hours and then declined remarkably. At 247 mOsm, survival diminished continuously, with a slope close to 1, while at 204 mOsm mortality did not reach 30 % after five days of the test.

Figure 2 shows the survival curves as a function of time for each of the NaCl solutions assayed at both densities. In this case also, the effect upon survival began to appear 48 hours after exposure, and after this time mortality increased continuously with time, but

Tab. 1 - Mean lethal osmolarity (LO 50) of mannitol and NaCl in premetamorphic *Bufo arenarum* tadpoles under two conditions of density (1 tadpole/4 ml and 1 tadpole/20 ml). Degrees of freedom: 3. $n = 40$ tadpoles/solution.

		time (h)	LO 50 (mOsm)	confidence limits	slope	correlation coefficient
mannitol	1 tadpole /4 ml	48	262.9	252.2-278.8	13.14	0.94
		72	245.8	236.7-256.8	13.42	0.97
		96	228.7	220.4-237.3	14.28	0.99
		144	221 5	208.8-233.4	14.64	0.99
	1 tadpole /20 ml	48	267.4	262.1-274.4	29.20	0.99
		72	241.2	234.5-247.5	21.23	0.99
		96	230.1	223.4-236.4	18.8	0.99
		144	224.6	215.0-234.9	20.16	0.99
NaCl	1 tadpole /4 ml	48	282.2	272.6-297.8	14.76	0.99
		72	249.4	243.9-255.2	17.46	0.98
		96	235.3	230.3-240.2	19.78	0.99
		144	215.1	209.5-220.2	17.94	0 99
	1 tadpole /20 ml	48	305.6	285.2-364.1	12.01	0.97
		72	274.8	265.6-289.8	15.83	0.98
		96	263.7	256.0-273.8	16.35	0.98
		144	220.6	211.1-228.5	12.83	0.99

differently to that observed with mannitol. The survival curve gradient between the two densities was different for 247 and 271 mOsm. At the end of the bioassay, however, values found were very similar for both densities. At 204 mOsm, the final mortality rate was around 30 %.

Table 1 shows the results of Probit analysis for NaCl and mannitol at the two assayed densities. Since the mean lethal osmolarity values (LO 50) for mannitol and NaCl show overlapping confidence limits for all the times of the assay and for both densities, we can conclude that, under these experimental conditions, the chemical composition and the high organism density have not effect on the survival response of *Bufo arenarum* tadpoles. This allows to calculate an LO 50 (mean value ± standard error) for 48 and 144 hours of 279.0 ± 9.6 and 220.5 ± 2.0 mOsm respectively. These values are higher than those recorded for anuran tadpole plasma (Degani & Nevo, 1986). Padhye & Ghate (1992) determined a mean lethal concentration (LC 50) of NaCl and KCl for different stages of embryos and tadpoles of *Microhyla ornata*. They reported a LC 96 of NaCl of 0.69 %; in hind limb tadpoles stage, a value very close to the one reported here. The results obtained show that *Bufo arenarum* premetamorphic tadpoles present a similar response as concerns survival in an environment highly osmotic which is independent of the electrolytes. The low levels of (Na + K)-ATPase detected in anuran tadpoles (Kawada et al., 1969) suggest that Na exchange with the medium is only passive (Warburg & Rosenberg, 1990); under the assay conditions, *Bufo arenarum* tadpoles are probably able to compensate the osmotic gradient by keeping their plasma slightly hyperosmotic with reference to the incubation medium through a relative increase of the NaCl (SHOEMAKER, 1992; BALINSKY, 1981). The values of LO 50 found in this study could indicate the possible limits of such a compensation.

ACKNOWLEDGMENTS

The results reported here were part of the author's Doctoral Dissertation carried out under the guidance of Dr. A. SALIBIÁN at the University of Buenos Aires. The technical cooperation of Roberto YOSHIHARA and Javier KATZ is acknowledged. This work received financial support from the CONICET and from the National University of Lujan.

LITERATURE CITED

ANONYMOUS, 1992 – *Standard methods for examination of water and wastewater* 18th ed. Washington, D.C., APHA-AWWA, WPCF: i-xxxiv + 8.1-8 83

ALVARADO, R. J. & JOHNSON, S. R., 1966. – The effects of neurohypophysial hormones on water and sodium balance in larval and adult bullfrog (*Rana catesbeiana*). *Comp. Biochem. Physiol.*, **18**. 549-561.

BALINSKY, J. B., 1981. – Adaptation of nitrogen metabolism to hyperosmotic environment in amphibian. *J. exp. Zool.*, **215**. 335-350.

DEGANI, G. & NEVO, E., 1986 – Osmotic stress and osmoregulation of tadpoles and juveniles of *Pelobates syriacus. Comp. Biochem. Physiol.*, 83 (A): 365-370.

FERRARI, L., 1995. – *Equilibrio hidromineral de larvas de* Bufo arenarum: *respuestas compensatorias al "stress" osmótico y al cadmio*. Doctoral Dissertation, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires: 1-162.

FINNEY, D. J., 1971 – *Probit analysis*. Cambridge University Press: 1-333.

GOSNER, K. L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

KATZ, U., 1987. The effect of salt acclimation on the uptake and osmotic permeability of the skin of the toad (*Bufo viridis*, L.). *J. Physiol.*, **82**: 183-187.

KAWADA, U., TAYLOR, R. E., Jr. & BARKER, S. B., 1969. – Measurement of Na-K-ATPase in the separated epidermis of *Rana catesbeiana* frogs and tadpoles. *Comp. Biochem. Physiol.*, **20**: 965-975.

KHER, A. I., 1994. – Density-dependent response in tadpoles of *Bufo arenarum* (Anura, Bufonidae) *Physis*, (B), **49**: 31-36.

MIRANDA, L. A. & PISANO, A., 1993 – Efecto de la densidad poblacional en larvas de *Bufo arenarum* producido a través de señales visuales. *Alytes*, **11** 64-76

MURRAY, D. L., 1990 – The effects of food and density on growth and metamorphosis in larval wood frogs (*Rana sylvatica*) from central Labrador. *Can. J. Zool.*, **68**: 1221-1226.

PADHYE, A. D. & GHATE, H. V., 1992 – Sodium chloride and potassium chloride tolerance of different stage of the frog, *Microhyla ornata. Herp. J.*, **2**: 18-23.

SHOEMAKER, V. H., 1992. Exchange of water ions and respiratory gases in terrestrial amphibians. *In* M E. FEDER & W. W. BURGGREN (eds.), *Environmental physiology of the amphibians*, Chicago, Univ. Chicago Press: 125-150

WARBURG, M. R. & M. ROSENBERG, 1990. - Ion and water balance and their endocrine control in the aquatic amphibians *In*: W HANKE (ed.), *Biology and physiology of amphibians*, Stuttgart & New York, Gustav Fisher Verlag: 385-403.

Corresponding editors: Ulrich SINSCH & Alain DUBOIS.